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Evaluation Of Antibacterial Efficacy Of Carica Papaya Leaf and Seed Extract Against Enterococcus faecalis

Nishitha Arun[1], Sindhu Ramesh[2*], Anjali Sankar[3]

[1,3]Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

[2]Professor, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

***Corresponding author:** Sindhu Ramesh, Professor, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

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ABSTRACT

Introduction: Carica papaya is a well known medicinal plant. Its medicinal value is especially attributed to the presence of secondary metabolites such as tannins, flavonoids, and saponins. Various parts of papaya are used as a cure for various diseases.

Aim: To determine the antimicrobial activity of papaya leaf and seed extract against endodontic pathogens.

Materials and Method: The antibacterial efficacy of C.papaya leaf and seed extract was determined using the zone of inhibition agar disk diffusion method. The Minimum Inhibitory Concentration was determined using broth dilution method.

Results: E.faecalis showed sensitivity for Papaya leaf, seed extract and calcium hydroxide. The zone of inhibition was significantly greater for Papaya leaf extract when compared to papaya seed extract and calcium hydroxide at 25µg/mL, 50 µg/mL and 100 µg/mL ($p<0.05$). Both papaya leaf and seed showed the highest zone of inhibition at 100µg/mL. The test results showed potent effects of the Papaya leaf extract with MIC at 25µg/mL and For papaya seed extract MBC at 50µg/mL for E.faecalis.

Conclusion: Carica papaya leaf and seed showed prominent antibacterial efficacy against E.faecalis. The minimum inhibitory concentration of C.papaya leaf extract was 25µg/mL whereas for C.papaya seed extract was 50µg/mL. C.papaya leaf extract showed significant antibacterial efficacy against E.faecalis when compared to C.papaya seed extract and Calcium hydroxide. There was no significant difference in antibacterial efficacy between papaya seed extract and calcium hydroxide against E.faecalis.

Keywords: *Carica papaya leaf extract, seed extract, calcium hydroxide, E.faecalis, disk diffusion, zone of inhibition, medical, health*

INTRODUCTION

The resistance of microbes to synthetic drugs has created a need to discover newer antimicrobial agents world wide. The ancient tradition of usage of plants and their various parts in prevention and treatment of various diseases are being recently revisited. Papaya belongs to the Caricaceae family consisting of four genera in the world. The genus Carica Linn. is represented by four species in India, of which Carica papaya Linn. is the most widely cultivated and best-known species. The papaya leaves, fruits, seeds and latex are being used for several industrial as well as medicinal purposes to maintain health. Papaya extract contains a broad range of phytochemicals such as vitamins, enzymes, minerals, polysaccharide, proteins, fats and oils, alkaloids, glycosides, lectins, sterols, saponins, flavonoids (1–3) (4,5)(2,3,6).

The antibacterial efficacy of Carica papaya extract has been studied to evaluate its potential as a natural antimicrobial agent. All the parts of C.papaya are used in treatment of various diseases and health conditions(7)(8–11)(12,13)(7). Papaya is a rich source of various minerals such as magnesium, potassium along with antioxidants such as vitamin C, A and E(14)(15–18)(14). It also contains phytochemicals such as alkaloids, flavonoids, sterols and other essential proteinases such as papain, caricain, chymopapain and glycyl endopeptidase 6 (19–22). The potential antimicrobial effects of papaya extract are attributable to its diverse bioactive constituents, which comprise papain, chymopapain, carpain, and flavonoids. In laboratory studies, these compounds have exhibited antimicrobial properties against specific bacteria.

Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (23).

Leaf extracts contain phenolic compounds like p-coumaric acid, kaempferol, protocatechuic acid, caffeic acid etc (24)(25–28)(24). These compounds have antimicrobial activity especially karpain which helps in lysis of the microbes(29)(2)(29). Papaya seeds have antibacterial properties and are effective against E. coli, Salmonella and Staphylococcus infections(30)(31–35)(30). Plant derived antimicrobial agents are effective and also concurrently eliminates the side effects associated with synthetic agents. The roots, stem, seed, leaves and fruits of various plants are used as extracts or decoctions for medicinal use.

The aim of the present study is to determine and evaluate the antibacterial property of Carica papaya seed and leaf extract against E.faecalis.

METHODOLOGY

Collection of plants

Carica papaya leaves and seeds were obtained from Tamil Nadu Horticulture Institute, Madhavaram, Chennai, Tamil Nadu. The leaves and seeds were washed thoroughly with water and shade dried at room temperature for 15 days. The dried leaves and seeds were powdered using a slender blender.

Preparation of extract

The extract was prepared by adding 300mg of the powdered leaf and seed to 500ml of ethanol and was placed in the beaker for 24 hours. It was further condensed and reduced by heating at 25-30 degree celsius. The extract was purified and boiled at 25 degree celsius for 10 minutes.

Antibacterial Activity- Zone of Inhibition

Antibacterial activity of the C.papaya leaf and seed was determined against E.faecalis. This activity was carried out on MHA agar to establish the zone of inhibition. Muller hinton agar was prepared and sterilized at 120 pounds for 45 minutes. The media was poured onto the sterilized plates and set aside to solidify. The test species were swabbed after the wells were cut with a well cutter. The plates were filled with a herbal mixture of various concentrations and incubated for 24 hours at 37°C. The zone of inhibition was assessed after the incubation period. Calcium Hydroxide was used as positive control.

Minimum Inhibitory Concentration

The MIC was determined by liquid micro dilution method. Various concentration C.papaya leaf and seed (25,50 and 100µg/mL) to each well added to 50 ul microbial inoculum. The MIC is defined as: the lowest concentration of C.papaya leaf and seed which inhibits the visible growth after incubation for 24 hours at 37 degrees.

RESULTS

Zone of inhibition

E.faecalis showed sensitivity for Papaya leaf, seed extract and calcium hydroxide. The zone of inhibition was significantly greater for Papaya leaf extract when compared to papaya seed extract and calcium hydroxide at 25µg/mL, 50 µg/mL and 100 µg/mL at a significance of p=0.001,0.00, p= 0.005,0.002 and p= 0.033,0.020 respectively. There was no significant difference between Carica papaya seed extract and calcium hydroxide at all three concentrations ($p>0.05$). Both papaya leaf and seed showed the highest zone of inhibition at 100µg/mL. There was no significant difference between the various concentrations in papaya leaf and seed extracts but calcium hydroxide showed significantly higher zones of inhibition at 50µg/mL and 100µg/mL when compared to 25 µg/mL ($p<0.05$).

Minimum Inhibitory Concentration

The test results showed potent effects of the Papaya leaf extract with MIC at 25µg/mL and For papaya seed extract MIC at 50µg/mL for E.faecalis.

DISCUSSION

Enterococcus faecalis is a facultative, gram positive anaerobe which is a commensal of the gastro-instestinal tract. It has the ability to cause opportunistic infections and has several mechanisms to sustain in unfavorable conditions. It has the ability to adapt to a wide range of temperatures such as 10-60 degree celsius, high alkalinity, low oxygen, high pH and poorly nourished environment(36).

E.faecalis is commonly associated with failed root canal treatment rather than the primary infections. The presence of post endodontic pain and reinfection is attributed to the occurrence of E.faecalis. The prevalence of E.faecalis is about 90% in such cases. E.faecalis is one of the most commonly evaluated biologic indicators with high resistance to antimicrobials(37,38)(39–42)(37,38)(5).

The pulp and seed of Carica papaya has been reported to show bacteriostatic activity against various enteropathogens such as Enterobacter cloacae, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus vulgaris, Klebsiella pneumonia and Pseudomonas aeruginosa(43)(44,45)(43).

Bioactive compounds from papaya leaf and root showed antibacterial activity against human pathogenic bacteria. These extracts were reported to have marked inhibition against gram positive bacteria when compared to gram negative bacteria(30)(46–49)(30).

In the present study Carica papaya seed and leaf extract showed pronounced antibacterial properties against E.faecalis. The presence of secondary metabolites like flavonoids, alkaloids, saponins, tannins, steroids and other metabolites are attributed to antibacterial, anti-inflammatory and antioxidant properties of these medicinal plants(50)(51,52)(4,53)(50). The medicinal value of Carica papaya is due to the presence of these metabolites in various parts of the plant such as leaf, seed, fruit and latex(54)(55)(54) .Saponins, which are vast group of glycosides are reported to have antimicrobial, anti-allergic, anti-diabetic, anti-malarial and anti-inflammatory properties whereas flavonoids and tannins that are complex phenolic compounds synthesized by plants possess anticancer, antibacterial, anti-viral, anti-diabetic and antiallergic properties(56)(57,58)(56)(59–61).

The limitations of the present study is that the antibacterial efficacy of Carica papaya was determined only against one endodontic pathogen. Further studies to determine the antimicrobial efficacy against other endodontic pathogens are necessary followed by evaluation of the cytotoxicity of these extracts.

CONCLUSION

Carica papaya leaf and seed showed prominent antibacterial efficacy against E.faecalis. The minimum inhibitory concentration of C.papaya leaf extract was 25µg/mL whereas for C.papaya seed extract was 50µg/mL. C.papaya leaf extract showed significant antibacterial efficacy against E.faecalis when compared to C.papaya seed extract and Calcium hydroxide. There was no significant difference in antibacterial efficacy between papaya seed extract and calcium hydroxide against E.faecalis.

TABLE 1

Concentration	Zone of inhibition of Papaya Leaf extract	Zone of inhibition of Papaya Seed extract	Zone of inhibition of Calcium hydroxide
25µg/mL	12.67±0.52aA	10.25±0.35bA	9.25±0.45bB
50µg/mL	12.33±0.38aA	10.67±0.45bA	10.35±0.32bC
100µg/mL	13.21±0.76aA	11.35±0.65bA	11.13±0.45bC

Table 1: Zone of Inhibition in mm of papaya leaf, papaya seed extracts at 25,50 and 100 µg/mL and control (Calcium hydroxide). Lower case superscript shows the significant difference between the 3 tested compounds. 'b'- shows a statistical significant difference between papaya seed extract and calcium hydroxide with papaya leaf extract at similar concentrations (along the row). Upper case superscript shows significant difference between the 3 different concentrations of the tested compounds (along the column). 'C'- significant difference between 50µg/mL and 100µg/mL of calcium hydroxide compared to 25 µg/mL of calcium hydroxide.

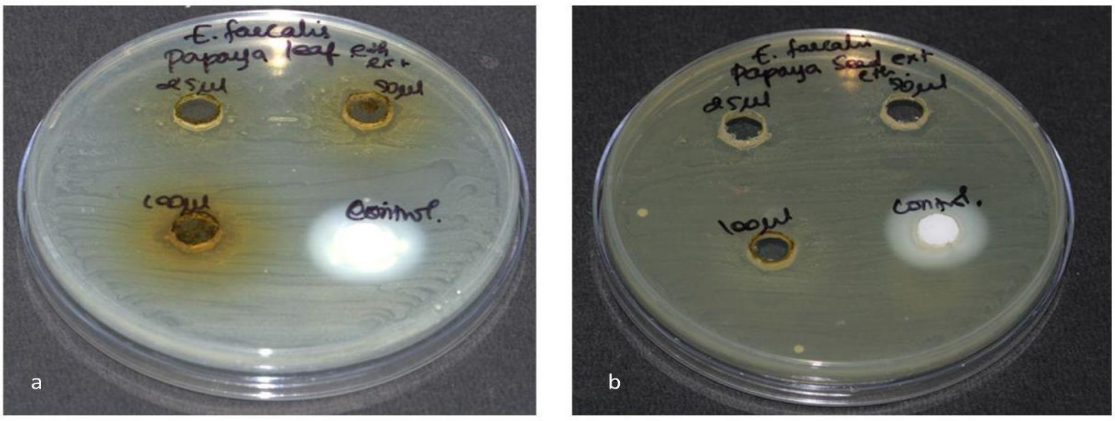

FIGURE 1: Zone of inhibition of E.faecalis in Agar Diffusion plate by 'a'- papaya leaf extract, 'b'- papaya seed extract at 25, 50 and 100µg/mL and control (Calcium Hydroxide).

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